Enhanced Protein Translation Underlies Improved Metabolic and Physical Adaptations to Different Exercise Training Modes in Young and Old Humans

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Summary

The molecular transducers of benefits from different exercise modalities remain incompletely defined. Here we report that 12 weeks of high-intensity aerobic interval (HIIT), resistance (RT), and combined exercise training enhanced insulin sensitivity and lean mass, but only HIIT and combined training improved aerobic capacity and skeletal muscle mitochondrial respiration. HIIT revealed a more robust increase in gene transcripts than other exercise modalities, particularly in older adults, although little overlap with corresponding individual protein abundance was noted. HIIT reversed many age-related differences in the proteome, particularly of mitochondrial proteins in concert with increased mitochondrial protein synthesis. Both RT and HIIT enhanced proteins involved in translational machinery irrespective of age. Only small changes of methylation of DNA promoter regions were observed. We provide evidence for predominant exercise regulation at the translational level, enhancing translational capacity and proteome abundance to explain phenotypic gains in muscle mitochondrial function and hypertrophy in all ages.
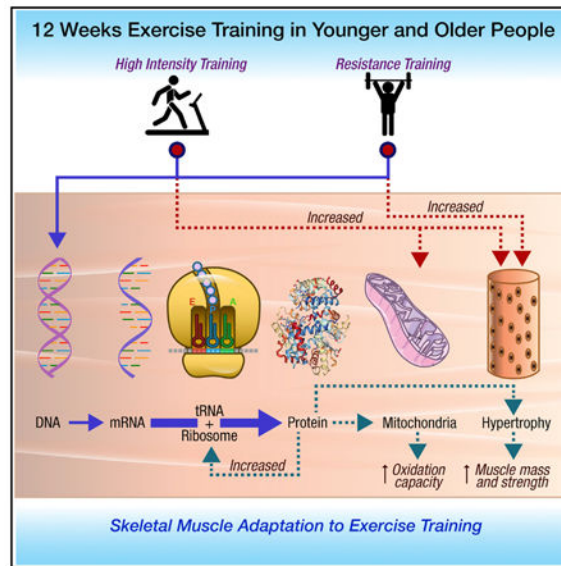
Graphical abstract

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12 Weeks Exercise Training in Younger and Older People

High Intensity Training   Resistance Training

Increased   Increased

DNA → mRNA → tRNA + Ribosome → Protein ⋯▶ Mitochondria   Hypertrophy

Increased

↑ Oxidation capacity   ↑ Muscle mass and strength

Skeletal Muscle Adaptation to Exercise Training

Introduction

Health benefits of exercise are indisputable in combating age-related risks for disease and disability (Myers et al., 2002), and understanding the transducers of such benefits is of high national interest (Neufer et al., 2015). Aerobic exercise training leads to skeletal muscle protein remodeling and stimulates multiple molecular steps,including DNA methylation (Barrè s et al., 2012) and synthesis of new proteins (Short et al., 2003). Many studies have demonstrated changes to mRNA content, but the extent to which transcriptional changes lead to changes in protein abundance remains inconclusive (Miller et al., 2016). Understanding the regulation of skeletal muscle molecular adaptations to diverse types of exercise training can help to develop future targeted therapies and exercise recommendations. There is a gap in knowledge about age effects on pathways regulating exercise adaptations in response to different exercise modalities.

Different types of exercise can stimulate variable, but specific, responses in muscle functions. Aerobic exercise training enhances mitochondrial oxidative enzymes' capacity (Holloszy, 1967) and coincides with improvements to insulin sensitivity with age (Lanza et al., 2008). It remains to be determined whether age-related decline in muscle mitochondrial protein synthesis (Rooyackers et al., 1996) is reversed by aerobic training. High-intensity aerobic interval training (HIIT) involves repeating short bouts of activity at near-maximal intensity, which rapidly and robustly increases aerobic capacity, mitochondrial respiration, and insulin sensitivity in young people (Burgomaster et al., 2008; Irving et al., 2011). Resistance training (RT) reverses sarcopenia and age-related declines in myosin heavy-chain gene transcripts and synthesis rates of muscle proteins (Balagopal et al., 2001), but a comprehensive gene transcripts and proteome comparison with aerobic training has not been performed. Combined training (CT) offers many benefits of both aerobic and resistance training, although the intensity of aerobic and resistance components are lower than either HIIT or standard RT programs (Irving et al., 2015). Lower exercise intensity may limit

training adaptations (Ross et al., 2015), particularly of mitochondria (MacInnis et al., 2016). A comprehensive approach to different exercise programs and the specific physiological and molecular adaptations and the potential impact of age on these adaptations remain to be determined.

We performed comprehensive metabolic and molecular phenotyping of young and older adults in response to 12 weeks of aerobic training (using HIIT), RT, and 12 weeks of a sedentary period followed by CT of moderate-intensity aerobic plus resistance training. These measurements were performed 72 hr following the last bout of exercise to specifically determine the training effect. We hypothesized that skeletal muscle transcriptome, translation, and proteome would increase with training, and the pattern of responses would reflect the type of training modality and phenotype changes.

HIIT robustly improved cardio-respiratory fitness, insulin sensitivity, mitochondrial respiration, and fat-free mass (FFM) in both age groups. RT improved FFM and insulin sensitivity in both age groups while CT had lesser gains, perhaps due to differences in training intensity. RNA sequencing of muscle biopsies revealed robust increases in mRNA expression with HIIT, more so than RT or CT, particularly of mitochondrial transcripts. Quantitative proteomics in response to HIIT revealed larger proteomic changes, particularly in mitochondrial and ribosome proteins, as well as reversal of many age-related changes. We report relatively small changes (<10%) to methylation of DNA promoter regions and low overlap between transcriptional and proteomic changes. Thus, our findings indicate that the regulation of exercise adaptation is tightly linked with protein translation and translational machinery.

Results and Discussion

Study Overview

The prospective exercise training study (Figure 1) was approved by the Mayo Clinic Institutional Review Board, registered at https://clinicaltrials.gov (#NCT01477164) and conducted in accordance with the Declaration of Helsinki. All participants provided informed written consent. Participants were recruited into two distinct age groups: young (18–30 years) or older (65–80 years) with a goal of an equal number of men and women. The final groups were approximately balanced for sex, and all women in the older group were post-menopausal. Exclusion criteria were structured regular exercise (>20 min, twice weekly), cardiovascular disease, metabolic diseases (type 2 diabetes mellitus, fasting blood glucose > 110 mg/dL, and untreated hypothyroidism or hyperthyroidism), renal disease, high body mass index (BMI > 32 kg/m^2), implanted metal devices, pregnancy, smoking, and history of blood clotting disorders. Exclusionary medication included anticoagulants, insulin, insulin sensitizers, corticosteroids, sulfonylureas, barbiturates, peroxisome proliferator-activated receptor γ agonists, β blockers, opiates, and tricyclic antidepressants.

Following baseline measurements, the participants were randomized to three groups (HIIT, RT, or CT) using gRand (v1.1, Peter A. Charpentier) following a permuted block strategy with block length of 15 and 2 factors (age and sex). HIIT was 3 days per week of cycling (4 3 4 min at >90% of peak oxygen consumption [VO$_{2\ peak}$] with 3 min pedaling at no load)

and 2 days per week of treadmill walking (45 min at 70% of $VO_{2\ peak}$). RT consisted of lower and upper body exercises (4 sets of 8–12 repetitions) 2 days each per week. CT participants first underwent a 12-week sedentary period (SED) and wore accelerometers to record any structured activity. Following SED, participants underwent metabolic studies and began CT of 5 days per week cycling (30 min at 70% $VO_{2\ peak}$) and 4 days per week weight lifting with fewer repetitions than RT. Both baseline and post-training studies were performed in all participants.

Baseline subject characteristics show that older participants had higher body fat percentage, BMI, and fasting plasma glucose concentrations despite similar fasting insulin concentrations (Table 1). During training, the weekly energy expenditure of exercise per FFM was highest with HIIT (Young: 26 ± 3; Older: 18.5 ±2, p < 0.001) followed by CT (Young: 22.8 ±2; Older 16.9 ± 1, p < 0.05) and lowest with RT (Young: 9.6 ± 2; Older 7.3 ± 1, p < 0.0001). All baseline comparisons are mean ± SD.

Cardio Respiratory Fitness, Muscle Mass, and Insulin Sensitivity Improve with Training

$VO_{2\ peak}$ during a graded exercise test was determined at baseline and following training. There was a high correlation ($r^2 = 0.988$, p < 0.0001) and low variability between pre- and post-SED $VO_{2\ peak}$ even though measurements were separated by 12 weeks (Young: Pre = 2,643 ± 649, Post = 2,517 ± 603; Old: Pre = 1,646 ± 567, Post = 1,627 ± 550 mL/min, Figure S7). The respiratory exchange ratio (RER) for SED group was also consistent for both young (Pre: 1.2 ± 0.1, Post: 1.2 ± 0.1) and older adults (Pre: 1.2 ± 0.1, Post: 1.2 ± 0.1), indicating that $VO_{2\ peak}$ measurements were done during identical conditions.

Compared to young, older adults had ~30% lower $VO_{2\ peak}$ relative to body weight (Figure 2A). Absolute $VO_{2\ peak}$ (mL/min) significantly increased in the younger group following HIIT (mean[95%CI]: +637[462–812] p < 0.0001) with lesser but significant increase with RT (+185[1–368] p = 0.048) and CT (+429 [223–634] p = 0.0001). In the older group, absolute $VO_{2\ peak}$ also increased following HIIT (278[72–483] p = 0.0091) and CT (+295[75–514] p = 0.0096); however, the increase in absolute $VO_{2\ peak}$ of the older RT group did not reach statistical significance (+203[−3–409] p = 0.053). In the young group, HIIT produced the highest increase of ~28% in relative $VO_{2\ peak}$ (+8.3 [6.2–10.3] p < 0.0001 mL/kgBW/min) followed by ~17% with CT (+5.3[2.9–7.6] p < 0.0001) (Figure 2B) without any significant increase with RT. In the older group, relative $VO_{2\ peak}$ increased ~17% with HIIT (+3.5[1.2–5.9] p = 0.0042) and ~21% with CT (+4.4[1.8–6.9] p = 0.0011) without any significant change following RT (+2.3[−0.1–4.6] p = 0.06) (Figure 2B).

Frailty with age is largely due to muscle wasting and weakness orsarcopenia(Goodpasteretal., 2006). Declines in FFM and muscle quality (e.g., force per muscle mass) with age contribute to decreased exercise capacity (Delmonico et al., 2009). We investigated the response of muscle mass and quality to different exercise modalities. Baseline whole-body FFM was similar between young and older groups (Figure 2C). Whole-body FFM increased in all training groups, with the greatest increase in young RT (2.2 kg; +4%, p < 0.0001; Figure 2D). Leg strength was lower in older humans in absolute terms or relative to leg FFM (Figure 2E, Young: 15.8±3.8, Older:13±4.1one-repetitionmaximum [1RM]/ kg leg FFM, p = 0.017), suggesting lower muscle quality with

age. The training groups with resistance training (RT and CT) had increased leg strength per change in leg mass, indicating an increase in the capacity for a given mass of muscle to produce force (Figure 2F). Leg strength did not change significantly with HIIT, possibly due to training specificity associated with cycling versus leg press exercises. Alternatively, the increase in strength was related to increase in muscle mass. These results demonstrate that both muscle strength and mass robustly improved with CT and RT in both younger and older adults. Collectively, the gains in whole-body FFM suggest that a high-intensity aerobic stimulus can induce both aerobic and hypertrophy adaptations.

Exercise intensity is a strong influence on adaptations. CT had lower-intensity aerobic and resistance components than HIIT and RT, respectively. Approaches to improve exercise responses will have positive benefits on public health, and raising exercise intensity can increase the number of exercise responders (Ross et al., 2015). A previous work in younger adults demonstrated that 12 weeks of HIIT increased $VO_{2\ peak}$ and muscle citrate synthase activity to a similar extent as longer duration of lower-intensity aerobic exercise training (Gillen et al., 2016). We demonstrate that HIIT is a feasible approach to increase exercise intensity in healthy younger and older adults. Younger adults demonstrated more robust increase of $VO_{2\ peak}$ in response to HIIT unlike older adults who responded equally to HIIT and CT (Figure 2B).

Older adults are at risk for developing insulin resistance associated with sedentary lifestyle and gains in adiposity (Karakelides et al., 2010). Exercise can improve insulin sensitivity, and we sought to clearly define the age effect on different types of exercise training and age on insulin sensitivity. For this, we measured peripheral insulin sensitivity as the glucose rate of disappearance (R_d [glucose rate of disappearance] μmol/ kgFFM/min) during a two-stage hyperinsulinemic-euglycemic clamp (mean ± SD steady state glucose was 88 ± 6 mg/dL) at baseline and after exercise training in all groups (Figures S1 and S3). At baseline, young and older adults had similar insulin sensitivity (Figure 2G). R_d increased in all training groups, except in older CT (Figure 2H). Fasting insulin and glucose did not change with training ineither age group (Figure S1). Predominant fates of glucose in skeletal muscle are either oxidative as fuel or non-oxidative for storage as glycogen. Non-oxidative glucose disposal increased with training (Figure S3), indicating greater storage rather than oxidation, which is a useful training adaptation for promoting exercise performance. These results are consistent with a previous cross-sectional study showing chronically trained older and younger adults have similar measurements of insulin sensitivity (Lanza et al., 2008). We did not detect any changes to hepatic insulin sensitivity (Figures S2 and S3), indicating that improvements were predominantly in skeletal muscle metabolism, suggesting that the previous cross-sectional data showing enhanced insulin sensitivity to endogenous glucose production represent long-term (4 years) exercise training effect (Lanza et al., 2008).

Mitochondrial Decline with Age and Improvement with Training

A major exercise effect to skeletal muscle metabolism is mitochondrial oxidative capacity. Declines in mitochondrial content with age are closely linked to reduced cardiorespiratory fitness (Short et al., 2005). Decreased resting mitochondrial ATP production has been implicated in the development of insulin resistance with aging (Petersen et al., 2003).

Indeed, a relationship between insulin-resistant states and decreased oxidative enzymes in skeletal muscle has been previously reported in obesity and type 2 diabetes (Simoneau and Kelley, 1997). However, this relationship is not always observed (Karakelides et al., 2010). We investigated aging and exercise training effects on isolated mitochondria from skeletal muscle biopsy samples collected in the resting and fasting state and then determined maximal mitochondrial oxygen consumption by high-resolution respirometry.

At baseline, maximal respiration was lower in older adults compared to young for the respiratory complexes (Complex I+II displayed in Figures 3A and 3C), expressed in either absolute units or normalized to mitochondrial protein content. HIIT increased maximal absolute mitochondrial respiration in young (+49%) and older adults (+69%), whereas a significant increase following CT was observed in young (+38%), but not older adults (Figures 3B and 3D). RT did not increase mitochondrial respiration significantly in either age group. The intrinsic functions of mitochondria, including coupling efficiency and reactive oxygen species production, were not different either between age groups or in response to training (Figures S4A–S4D). Older adults had lower mtDNA copy number when normalized to nDNA, consistent with a decline in mitochondria content with age (Figure S4E). HIIT and RT increased mtDNA content in older adults, with non-significant gains following CT (Figure S4F).

Collectively, the mitochondrial data in our cohort of sedentary, but otherwise healthy, adults indicate that a change in mitochondrial protein content was a predominate contributor to the loss of mitochondrial respiratory capacity with age and gains with training. There was no difference in insulin sensitivity at baseline despite differences in mitochondrial respiration. These results are in agreement with our previous work showing that differences in insulin sensitivity are more related to changes in exercise status and adiposity rather than mitochondrial capacity (Karakelides et al., 2010). Insulin resistance is associated with decreased mitochondrial respiratory chain efficiency and increased reactive oxygen species (ROS) production (Anderson et al., 2009), which can be restored in insulin-resistant women by aerobic training to those of a lean phenotype (Konopka et al., 2015). Our current study of healthy older adults with insulin sensitivity similar to younger adults showed no difference in respiratory chain efficiency or ROS production despite lower mitochondrial capacity than the younger group, supporting a notion that reduced insulin sensitivity is not associated with reduced mitochondrial coupling efficiency.

Exercise Training Enhances Skeletal Muscle Gene Expression Regardless of Age

We investigated the extent to which changes in mRNA coincided with phenotypes to further understand the regulation of skeletal muscle changes with age and adaptations to exercise. We performed RNA sequencing on baseline and post-exercise training skeletal muscle biopsies to assess whether transcript levels account for aging or training phenotypes of mitochondria, muscle hypertrophy, and insulin sensitivity. At baseline, when compared to young, 267 gene transcripts were lower and 166 were higher in older people (Figure S5A). Several mitochondrial-, insulin signaling-, and muscle growth-related genes were downregulated with age (Figure S5A). In contrast, among all training regimens, HIIT increased the expression of the largest number of genes in both young and older, especially

in mitochondrial, muscle growth, and insulin signaling pathways in older adults (Figures 4A and 4B). Older HIIT increased 22 mitochondrial genes, including those involved with translational regulation (ribosomes *MT-RNR1* and *2*) and mitochondrial tRNA transferase for methionine (*MT-TG*), leucine (*MT-TL1*), valine (*MT-TV*), glycine (*MT-TG*), and arginine (*MT-TR*). When compared to HIIT, RT increased 35% and 70% fewer genes in young and old, respectively (Figures 4C and 4D), and CT increased 28% and 84% fewer genes in young and old, respectively (Figures 4E and 4F). These data demonstrate a varied response of gene transcripts based on exercise mode between young and older adults, and the greatest increase was following HIIT in older adults.

Next, we determined whether training-induced gene sets are specific to training modes in young and older adults. The young had 274, 74, and 170 genes uniquely increased by HIIT, RT, and CT, respectively (Figure 4G). The older had 396, 33, and 19 genes uniquely increased by HIIT, RT, and CT, respectively (Figure 4H). Taken together, these data show that HIIT induced the largest gene expression change regardless of age. In older adults, the changes in gene expression with HIIT completely subsumes CT and RT changes. Given that older HIIT produced the largest gene expression change, we assessed whether these genes were unique or overlapping with the younger training groups. One-third of the older HIIT genes (181 out of 553) were also shared by the young HIIT group, and 114 of these were shared with young RT and CT groups (Figure 4I). Another third of older HIIT genes were unique to that group (186 out of 553; Figure 4I). Taken together, these data suggest that a large portion of older HIIT genes is age specific.

HIIT had a robust effect on increasing gene transcript content, and we next considered whether training in older individuals reversed age-related loss of muscle gene transcripts, potentially contributing to changes in metabolic phenotypes. To test this, we rank ordered young versus old baseline gene transcript changes from most upregulated to most downregulated with age. A gene set enrichment analysis (GSEA) was performed using genes that were upregulated with either old HIIT (Figure 4J) or young HIIT (Figure S5B). We observed that a majority of genes that were upregulated with HIITinboth groups also were upregulated with age (enrichment false discovery rate [FDR] = 0.0169 for old HIIT and FDR < 0.0001 for young HIIT). Table S1 shows the expression relationship of individual genes that were different with age at baseline and then changed after HIIT inthe old. These data support the hypothesis that HIIT is more likely to enhance expression of genes that are also increased with age. There were 11 genes that were significantly decreased in older adults and then were upregulated in older HIIT (Table S1), indicating that HIIT reversed a few specific genes that were decreased with age. Collectively, the gene overlap and GSEA demonstrate that exercise training did not reverse all the age-related declines in gene transcripts per se but induced specific patterns of genes in both young and older adults.

Finally, we were interested in whether there is a common group of genes that are upregulated in all exercise training types and both age groups (i.e., a universal gene set induced with training). A total of 55 genes were upregulated across all training types and in both age groups (Table S2). Gene ontology analysis revealed that these genes are primarily involved in angiogenesis and regulation of angiogenesis (Figure 4K; Table S3). An upstream regulator analysis of the universal gene set identified major transcriptional regulators, like

vascular endothelial growth factor, angiotensinogen, fibroblast growth factor, and interleukin 10-receptor subunit (Figure 4L; Table S4). Taken together, the universal exercise training gene set involves cardiovascular remodeling across training and age groups.

Skeletal Muscle Methylation Is Not Significantly Affected by Training

We wanted to test whether the observed exercise training-induced transcriptional changes are related to methylation of DNA. Previous studies show that acute bouts of exercise can alter DNA methylation (Barrès et al., 2012) and influence mRNA expression; yet, the effects of exercise training are less known. Global DNA methylation analysis was performed at baseline and after exercise training in all groups. At baseline, a total of 3,874 promoter CpG sites were differentially methylated between young and old groups (Figure S6). However, we observed statistically insignificant changes in gene promoter methylation due to exercise training in both age groups (Figure S6). These data show that the large gene expression changes observed after 12 weeks of training are not fully explained by a concurrent change in gene promoter methylation.

Previous work was able to detect an ~10% ($p < 0.05$) decrease in methylation of DNA within 20 min of acute exercise (Barres et al., 2012). Nitert et al. also reported changes to DNA methylation was altered within 48 hr after exercise following 6 months of lower-intensity aerobic training in middle age adults (Nitert et al., 2012). The acute changes to methylation coincide with time course studies showing mRNA content peaks within several hours after exercise followed by a general return to baseline at 24 hr (Louis et al., 2007). The current study demonstrated relatively small changes (<10%) in DNA methylation in comparison to a more substantial increase in mRNA content following exercise training, while studies by others demonstrate changes to DNA methylation in select genes using either shorter times to biopsy sampling (Barres et al., 2012) or longer training interventions (Nitert et al., 2012). We cannot exclude the possibility of acute changes to DNA methylation, as shown by others (Barres et al., 2012), that may contribute to dynamic changes in transcription, but our results show no robust differences in DNA methylation by 72 hr.

Training Induces Proteome-wide Expression Changes in Skeletal Muscle

Exercise exerts widespread influence on many muscle proteins (Lanza et al., 2008), yet there is controversy regarding the transcriptional and translational regulation of exercise adaptations. Exercise-induced mRNA do not necessarily translate into proteomic changes (Miller et al., 2016). Hence, we were interested in determining the overlap between mRNA and protein abundance at 72 hr post-exercise. The CT group was not included in the analysis due to attenuated gene expression changes. An intensity-based, label-free proteomics analysis was performed to detect differentially expressed proteins at baseline and post-intervention muscle samples, with significance at an adjusted p value   0.05 and absolute log2 fold change   0.5. The SED group had low variability in the control period with only five upregulated proteins, supporting that the changes that we observed in the exercise groups were not time-related changes but occurred in response to exercise. We also considered the pathways of genes and proteins that were expressed with exercise training to provide insight into potential regulatory mechanisms.

Baseline proteomic analysis revealed lower protein abundance in older adults for many proteins but specifically of 33 mitochondrial proteins (Figure 5A), which is consistent with the decreased mitochondrial respiratory capacity in the older group at baseline. Mitochondrial protein abundance increased following RT in both young and old (Figures 5B and 5C), but HIIT produced the largest increase in protein abundance particularly in the older (Figures 5D and 5E), which was consistent with the large changes in gene transcripts in the older with HIIT. HIIT in the older group induced pathways that are reflective of an oxidative phenotype, while both HIIT and RT induced pathways related to protein translation, including aminoacyl-tRNA biosynthesis and tRNA aminoacylation (Table S5). Of the proteins that changed with training, only 35 in the younger and 38 in the older were simultaneously upregulated in both HIIT and RT (Figures 5F and 5G). The gains in mitochondrial protein abundance occurred despite relatively lower changes in mRNA (compare to Figures 4B–4D) and demonstrate a dissociation between mRNA and protein abundance.

Our data suggest that exercise training in older humans can induce a strong upregulation of mitochondrial proteins, predominantly with HIIT. Also, RT regulated a different set of proteins than HIIT in the older group, which is in contrast with high overlap (Figure 4H) between genes induced in older HIIT and RT. Thus, there was a dissociation between proteomic changes compared to the transcriptome. Combined with the increase in protein synthesis rates and overall translational machinery, our data indicate a robust post-transcriptional regulation of protein abundance with exercise training. The common pathways that were induced in all training groups have important roles in protein translation, including tRNA amino acylation and branched-chain amino acid synthesis, as well as upregulation of ribosomal proteins. Collectively, these increases are consistent with increased protein translation capacity, and we next considered whether changes to mRNA were consistent with changes to protein abundance.

We considered whether changes to the synthesis rate of mitochondrial proteins could contribute to the changes in mitochondrial proteome, respiration, or DNA content. We used a stable isotope tracer-based approach to measure protein synthesis rates of skeletal muscle mitochondria. At baseline, there was no difference in protein synthesis rates between young and older groups (Figure 3E). HIIT increased ($p < 0.05$) mitochondrial protein synthesis in both young and older. RT and CT also increased mitochondrial protein synthesis in older, but not younger, adults (Figure 3F). The increase in mitochondrial protein synthesis across training groups in older people demonstrated that mitochondrial adaptations occurred with both aerobic and resistance training protocols. The increase in protein synthesis rates indicates greater translation of mitochondrial proteins and is consistent with results of mitochondrial proteome.

Of the 553 mRNA and 267 proteins that increased with HIIT in the older adults, only 12 were increased at both the mRNA and protein levels. The discrepancy indicates that changes in mRNA do not necessarily lead to changes in protein abundance. At the mRNA level, genes involved in translation to proteins and protein catabolism were significantly downregulated after training in older HIIT (Figure 6A). Yet, at the protein level, pathway analysis revealed that proteins involved with the mitochondrial envelope and mitochondrial

biogenesis were increased with HIIT (Figure 6B). A list of additional mitochondrial pathways is included in Table S6. Previous work in younger men identified 31 differentially expressed proteins that are involved in respiration and citric acid cycle following 7 days of aerobic training (Egan et al., 2011). Our results provide evidence that gains of the mitochondrial proteome occur in response to both aerobic and resistance training, and the response to training persists with longer training duration involving upregulation of the translational machinery, including ribosomal proteins, mitochondrial organization, and biogenesis (synthesis). Of interest, these changes occur robustly in older adults who have reduced mitochondrial biogenesis (Rooyackers et al., 1996) and proteome abundance (Short et al., 2005).

The dissociation between proteome and transcriptome may be explained on the basis of many factors. There are technical issues in comparing mass spectrometry-based proteome and RNA sequencing-based transcriptome with variable sensitivity and precisions of these measurements. Many transcriptomes that might enhance following acute exercise bouts may contribute to translation of proteins, and those transcripts may not show increase at 72 hr following exercise bout. In other words, the half-life of transcriptome and proteome may be different. Moreover, protein abundance is also influenced by the degradation of proteins. Indeed, resistance exercise increases activation of the ubiquitin proteasome and autophagy lysosome pathways that regulate protein degradation in young and older adults, particularly within several hours after exercise (Fry et al., 2013). However, measuring the activation of such pathways does not provide information into which individual proteins are being degraded. We cannot exclude the possibility that changes to individual protein degradation occurred with exercise and therefore may influence the dissociation between mRNA content and protein abundance.

At the protein level, ribosomal proteins were significantly upregulated in old HIIT and, to a lesser degree, in old RT (Figure 6C; Table S7) and provide a mechanism to contribute to the increased mitochondrial protein synthesis. Increases in protein synthesis, and subsequently improved protein turnover, may provide a protective effect against accumulation of proteins with irreversible post-translational modifications. Consistently, there was significantly lower protein oxidation and deamidation observed after training in old HIIT and old RT when compared to the old SED group (Figure 6D). All of these data support the hypothesis that the changes observed in protein abundance after exercise training were most likely due to translational regulation rather than transcriptional.

Conclusion

We assessed the effects of three different exercise modalities on skeletal muscle adaptations in young and older adults and explained on the basis of changes in transcriptome, translational regulation, and proteome abundance. HIIT training in young and older adults increased $VO_{2\ peak}$, insulin sensitivity, mitochondrial respiration, FFM, and muscle strength. In contrast, RT increased insulin sensitivity and FFM, but not $VO_{2\ peak}$ or mitochondrial function. CT involved lower intensity than HIIT or RT groups and resulted in modest gains in FFM and $VO_{2\ peak}$, with modest gains in insulin sensitivity, primarily in young people.

Supervised HIIT appears to be an effective recommendation to improve cardio-metabolic health parameters in aging adults.

We were interested in understanding the molecular transducers of exercise adaptations and performed RNA sequencing to determine changes to gene transcripts in skeletal muscle biopsies. HIIT robustly increased gene expression, particularly in older adults, while RT and CT had less pronounced effects in both age groups. Of interest, a set of gene transcripts were increased with HIIT in both young and older groups despite select genes having either greater or lower content at baseline in older adults. These data demonstrated that HIIT induced a pattern of gene expression regardless of age. Finally, HIIT also had robust increases in transcriptional and translational regulation of muscle growth and mitochondrial pathways.

Our study was powered to detect relevant effect sizes at the proteomic level, which demonstrated robust gains, particularly in proteins regulating translation. There were also robust effect sizes for training groups on metabolic phenotypes. For example, HIIT training in older adults had strong effect sizes in multiple outcomes, including mitochondrial respiration (1.7), aerobic fitness (0.99), insulin sensitivity (0.5), and smaller effect sizes for 1RM leg press (0.3) and FFM (0.1). Other parameters, such as DNA methylation, did not detect differences, and we cannot exclude the possibility of type II error. Additionally, a source of variability between mRNA content and protein abundance is the potential for splice variants to generate peptides that may not be annotated in mass spectrometry libraries. A high degree of alternative splicing could impact our datasets and potentially underestimate the relationship between protein abundance and mRNA content, as multiple splice variants may lead to the same peptide.

The increases in specific muscle proteins were greater relative to changes in mRNA content, particularly in mitochondrial and ribosomal proteins, and demonstrate a lack of direct relation between transcriptional and proteomic adaptations. DNA methylation is a regulatory point for transcription and had relatively small changes. Collectively, these data suggest that exercise adaptations are regulated to a greater extent at the post-transcriptional level. Increased ribosome protein content and other proteins involved in the translational machinery were detected following HIIT and provide for increased translational capacity. Mitochondrial protein synthesis was increased with HIIT as directly measured by isotope incorporation (representing translation). These data demonstrate an increase in both the protein translation machinery and synthesis rates of proteins. We also found a lowering of post-translational protein damages (oxidation and deamidation) following exercise training that may improve the functional quality of proteins. The increased mitochondrial protein synthesis, along with proteomic gains, despite differences in mRNA transcripts, support the hypothesis that translational level regulation is a predominant factor of mitochondrial biogenesis in human in response to exercise training. Further support for the above notion is provided by the increase in ribosomal protein content despite a fall in ribosomal transcript levels. The increases in specific proteins in muscle were greater relative to the changes in mRNA content, particularly in mitochondrial proteins and ribosomal proteins, and this demonstrates a lack of direct relation between transcriptional and proteomic abundances when measured 72 hr following the last bout of exercise. Together, the current results

demonstrated a predominant regulation of exercise adaptations at the post-transcriptional level.

Experimental Procedures

Study procedures are summarized here and detailed in the Supplemental Experimental Procedures.

VO$_{2 \text{ peak}}$ and Body Composition

VO$_{2 \text{ peak}}$ was measured with indirect calorimetry (Medgraphics Diagnostics) and an electronically braked cycle ergometer (Lode Medical Technologies). VO$_{2 \text{ peak}}$ was defined as reaching a perceived exertion > 17 on the Borg scale with RER > 1.1 (mean ± SD [Range] for Young: 1.2 ± 0.1 [1.1–1.43]; Older 1.2 ± 0.1 [0.98–1.36]) and achieving a heart rate within 10% of age-predicted maximal heart rate. Body composition was measured after an overnight fast with dual-energy X-ray absorptiometry as previously described (Nair et al., 2006).

Exercise Training

A 3-month, supervised, exercise-training program was conducted at the Dan Abraham Healthy Living Center. The Supplemental Experimental Procedures include the complete exercise list. 1RM leg press was determined as previously described (Irving et al., 2015). Aerobic exercise training zones were prescribed as the heart rate at a percentage of VO$_{2 \text{ peak}}$ and maintained ±5 beats/minute. VO$_{2 \text{ peak}}$ was measured during week 6 in HIIT and CT groups to increase training zones due to gains in aerobic fitness.

Metabolic Measurements

All pre- and post-intervention metabolic measurements were performed after 3 days of weight-maintenance meals (20% protein, 50% carbohydrates, and 30% fat). Participants refrained from exercise for 72 hr prior to metabolic study days.

Insulin Sensitivity—Hepatic and peripheral insulin sensitivity was measured using a two-stage euglycemic clamp (85–95 mg/dL) with somatostatin (Rizza et al., 1981). Glucose kinetics were determined using [6,6]-^{2}H2-glucose and indirect calorimetry (Lalia et al., 2015). Insulin was infused in two 3 hr stages at low and high rates (0.62 and 2.3 mU/kg FFM/min, respectively). Plasma glucose, tracer enrichment, and hormone concentrations for both age groups are provided (Figures S1–S3). Hepatic insulin sensitivity was computed as suppression of endogenous glucose production from basal to low insulin infusion. Peripheral insulin sensitivity was computed as glucose disposal during the final 60 min of the high-insulin stage.

Muscle Protein Synthesis—Fractional synthesis rate (FSR) of muscle proteins from biopsies of the current cohort was measured using isotopic tracer methodology. Participants were fasted overnight and a resting biopsy of the vastus lateralis (with 2% lidocaine) was collected at 0700 hr followed by a primed continuous infusion of *ring*-[^{13}C$_6$]-phenylalanine (1.5 mg/kg FFM prime and 1.5 mg/kg FFM/hr infusion). Biopsies were collected at 1,000 hr

and 1,500 hr during isotopic steady state, and FSR (%/hr) was calculated as $[\Delta E/(E_p \times t)] \times 100$, where t was hours between biopsies, ΔE was the tissue protein enrichment between biopsies (ΔE), and E_p was tissue fluid precursor enrichment.

Mitochondrial Function—Mitochondria were isolated from the 0700 hr biopsy and analyzed by high-resolution respirometry (Lanza and Nair, 2009). Mitochondria were added to a 2 mL chamber (Oxygraph-2K, Oroboros) followed by sequential additions of glutamate, malate, ADP, succinate, and inhibitors. Mitochondrial membrane integrity was verified with cytochrome-c.

Omics Measurements and Bioinformatics

Gene Expression Analysis—We measured mRNA expression as previously described (Lanza et al., 2012) with slight modifications. In brief, total RNA was isolated from the biopsy collected at 1,000 hr, and sequencing libraries were prepared with TruSeq RNA Sample Prep Kit v2. Libraries were sequenced on a HiSeq 2000 sequencer using TruSeq SBS sequencing kit version 3 and HCS version 2.0.12.0 software. Genes with a FDR-corrected p value of ≤ 0.05 and an absolute log2 fold change of ≥ 0.5 (where 0.0 signifies no change) were considered for further analysis.

Proteomics—Proteins were extracted from the 1,000 hr muscle biopsy sample and were subjected to liquid chromatography-tandem mass spectrometry (LC-MS/ MS) (Lanza et al., 2012). Label-free LC-MS/MS data were acquired using a high-resolution QExactive MS (Thermo Fisher Scientific). A total of 9.3 million MS/MS were collected from all analyses. MaxQuant software (v.1.5.1.2) was configured to match MS/MS against RefSeq human sequence database (v.58) and identify peptides and proteins at 1% FDR. Between two experimental groups, protein groups with a differential expression (corrected) p value of ≤ 0.05 and an absolute $\log_2$ fold change (FC) of ≥ 0.5 were considered for further analysis. Sample level protein oxidation and deamination were quantified by obtaining peptide-wise ratios of modified to unmodified (Mod/UnMod) intensities (Martin et al., 2016).

Methylation Analysis—Methylation analysis on the 1,000 hr skeletal muscle samples was performed using Infinium 450K array (Illumina). Raw chip data were processed using GenomeStudio (v.2011.1) to remove background, normalize for chip-to-chip variation, and compute β (% methylation) values for each CpG site. Partek Genomics Suite (v.6.13.0612; Partek) analyzed the CpG beta values between any two groups using ANOVA models. CpG sites that had a corrected p value ≤ 0.05 and an absolute Δβ value of ≥ 0.05% were considered as significant. CpG sites that were within the promoter regions (defined as 2,000 bp ≤ transcription start site ≤ 500 bp) of knowngenes were considered for further analysis.

Pathway and Gene Set Enrichment Analysis—Genes or proteins that were statistically up- or downregulated with different phenotypes (age at baseline and exercise training) were subjected to pathway analysis using WEBGESTALT software. Genes that were upregulated with all exercise training types in both age groups ("universal exercise response gene set") were subjected to Gene Ontology (GO) process enrichment using MetaCore software (Thompson Reuters) and upstream regulator analysis using Ingenuity

Pathway Analysis (QIAGEN). Gene set enrichment analysis was performed using Broad's GSEA software. All enrichment p values were FDR corrected (using Benjamini-Hochberg procedure), and entities with p values 0.05 were reported.

Statistics

Metabolic metrics were analyzed using JMP 10 (SAS Institute). Continuous baseline metrics between young and old groups were compared using two-way ANOVA (age × training). The SED group served asa no-treatment control, and pre- and post-intervention values were compared with a paired t test and p values from post hoc comparisons in the ANOVA models were corrected with Tukey's procedure. Training effect (HIIT, RT, and CT)onmetabolic metrics was evaluated by computing delta change in the metrics with intervention and comparing them using two-way ANOVA (age × training). Metabolic data in graphs are shown as least-squares adjusted means (type III population estimates) with 95% confidence intervals corrected with Tukey's procedure. The power was based on a two-sample t test with 20 per exercise training group (60 in total). The sample size was estimated a priori from a cross-sectional comparison of myosin synthesis rates that decline with age (mean [SD] for young, 0.64 [0.3]; older, 0.36 [0.4]). The detectable effect size for two-sided test with $\alpha = 0.05$ and 80% power was 0.9 SD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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In Brief

Robinson et al. assessed the effects of three different exercise modalities on skeletal muscle adaptations in young and older adults. While all enhanced insulin sensitivity, only HIIT and combined training improved aerobic capacity, associated with enhanced translation of mitochondrial proteins. HIIT effectively improved cardio-metabolic health parameters in aging adults.

Highlights

- High-intensity interval training improved age-related decline in muscle mitochondria

- Training adaptations occurred with increased gene transcripts and ribosome proteins

- Changes to RNA with training had little overlap with corresponding protein abundance

- Enhanced ribosomal abundance and protein synthesis explain gains in mitochondria
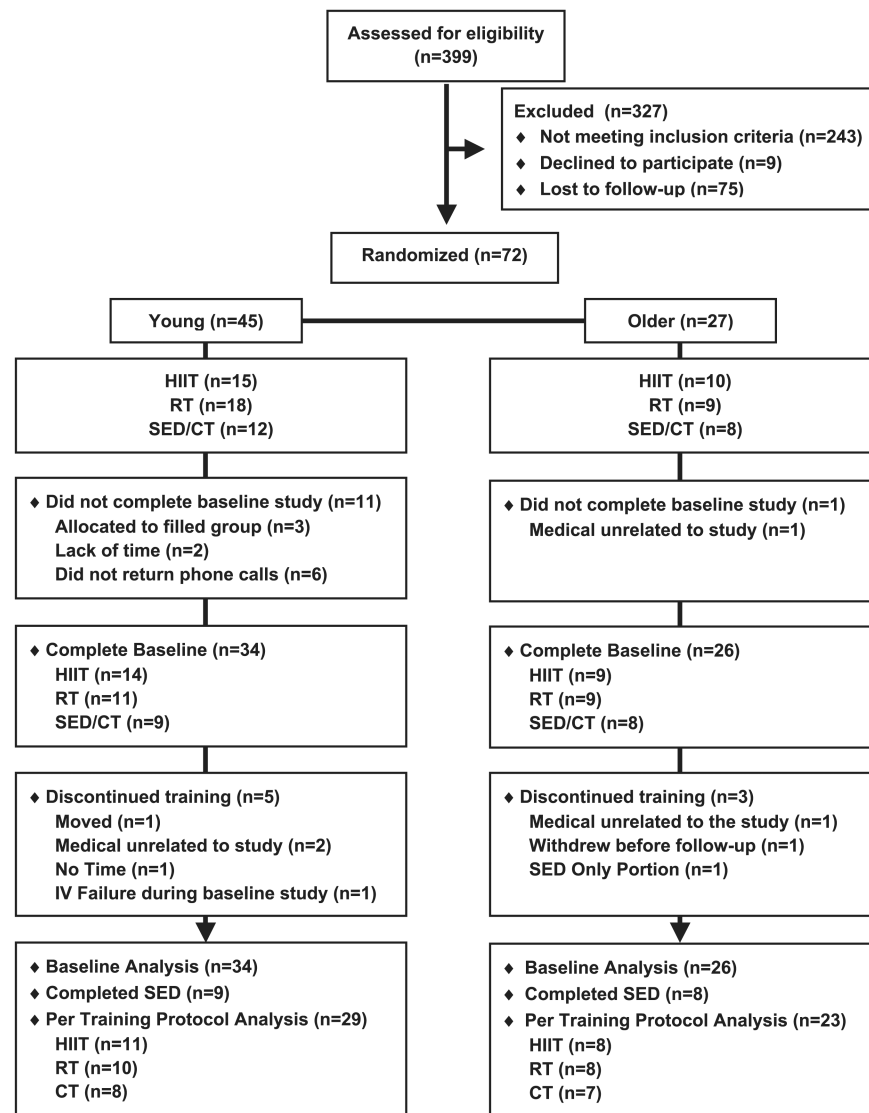
Figure 1. Study Recruitment

Study recruitment flow chart and final group sizes for high-intensity aerobic interval training (HIIT), resistance training (RT), or combined training (CT) that included a 12 week sedentary control period (SED). Five young adults dropped out of study due to time constraints (2), health unrelated to study (2), and IV failure (1). Three older adults dropped out due to health unrelated to study (1), did not want to perform follow up testing (1), and completed sedentary-only portion (1).
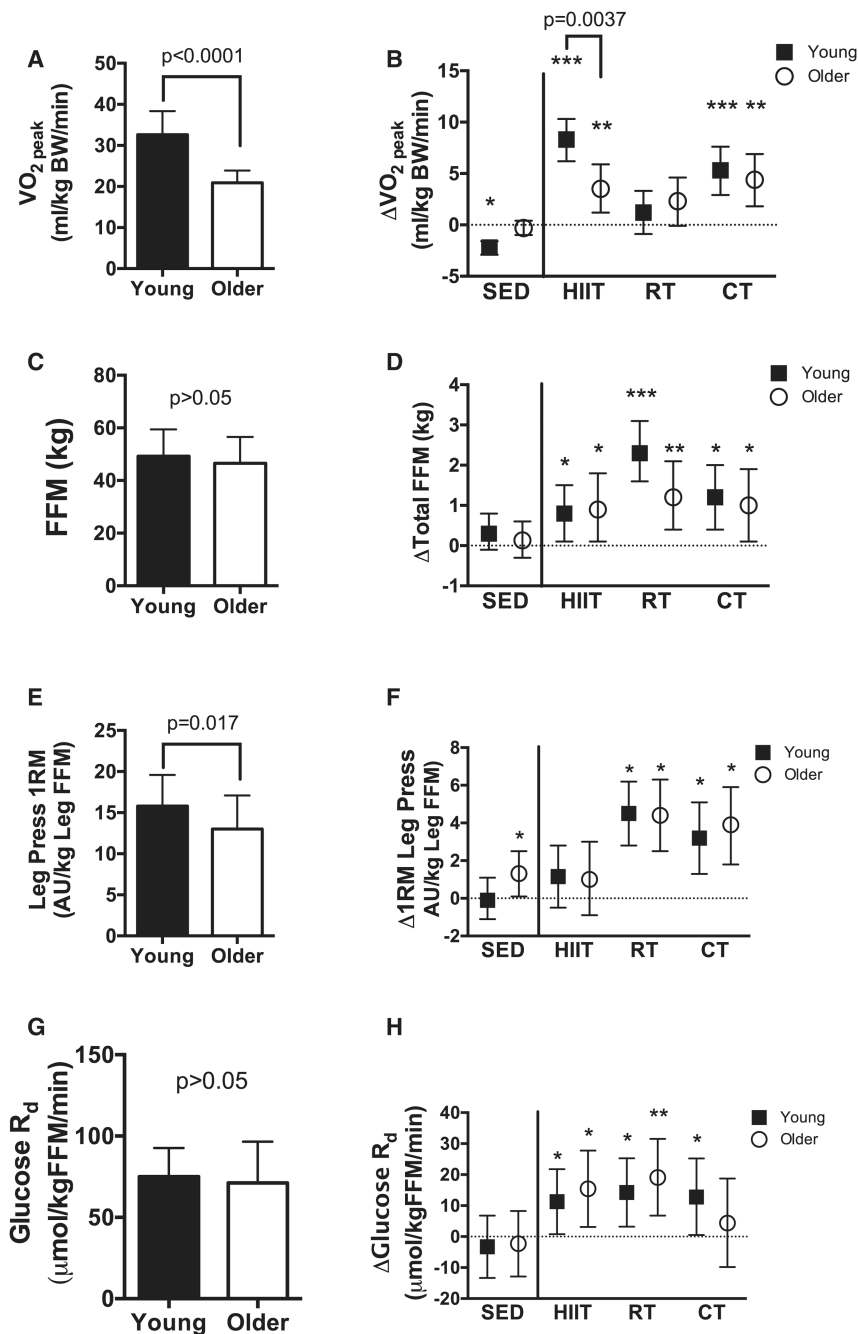
Figure 2. Baseline and Training Differences in Aerobic Fitness, Skeletal Muscle Mass, and Insulin Sensitivity

(A) Peak aerobic capacity per kg body weight ($VO_{2\ peak}$) was measured by indirect calorimetry during a graded exercise test and was lower in older adults at baseline.

(B) Exercise training improved the aerobic phenotype with pronounced gains following 12 weeks of aerobic training using high-intensity interval training (HIIT) compared to resistance training (RT) or combined training (CT) with sedentary (SED) control period.

(C) Fat-free mass (FFM) was measured by dual-energy X-ray absorptiometry and was similar between age groups at baseline.

(D) FFM increased in all training groups, particularly in young RT.

(E) Maximal leg strength, as 1 repetition maximum (1RM) leg press, was lower in older adults at baseline.

(F) 1RM increased in all training groups, with greatest gains following RT and CT.

(G) Insulin sensitivity was measured by a hyper-insulinemic-euglycemic clamp. The glucose rate of disappearance (R_d) during hyperinsulinemia was similar between age groups at baseline.

(H) Glucose R_d increased across training group with non-significant changes following CT in older. Changes during SED were analyzed separately and included in graphs for comparison. Data from baseline comparisons are displayed as mean ± SD with p values for unpaired t test. Changes with training are presented as least square adjusted mean with Tukey honest significant difference (HSD) 95% confidence intervals with the horizontal dotted line set at zero (no change from baseline). Within a training group, a difference between young and old is displayed as exact p value. Statistical significance from baseline is indicated as *p 0.05; **p 0.01; ***p 0.001. Exact p values are reported in Table S8.
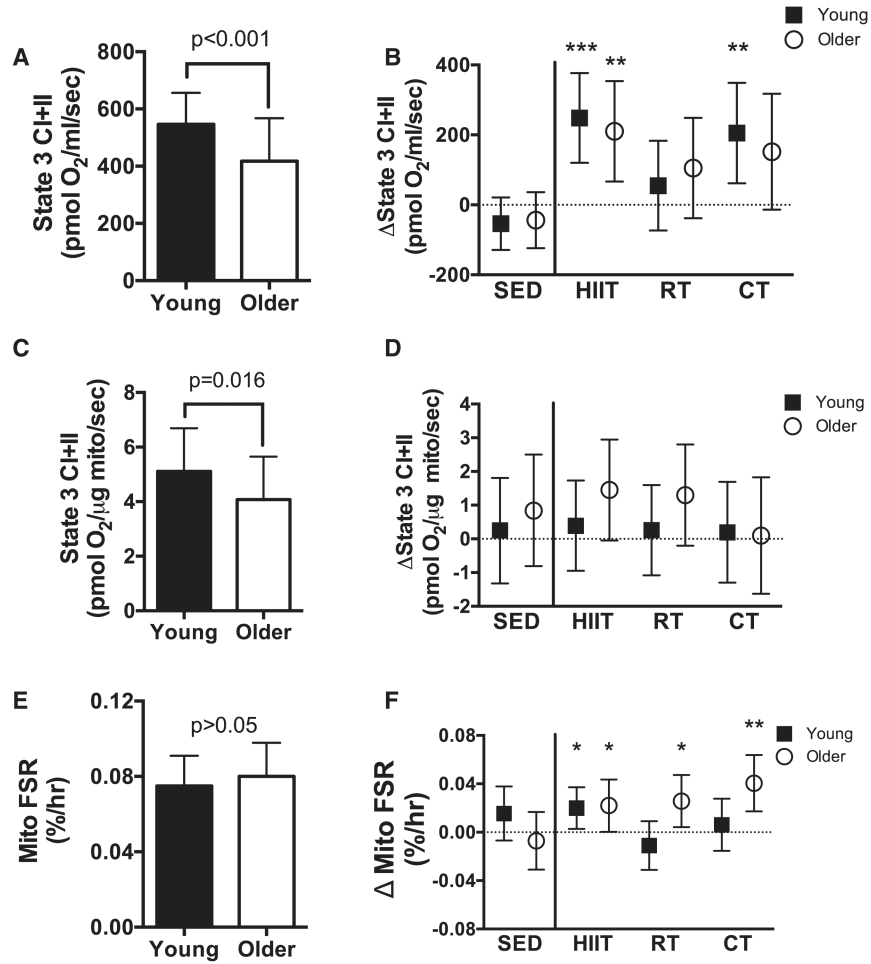
Figure 3. Baseline and Training Differences in Skeletal Muscle Mitochondria Respiration and Protein Synthesis Rates

(A) Mitochondrial respiration was measured using high-resolution respirometry and was lower per tissue content in older adults at baseline.

(B) Mitochondrial respiration per tissue increased with HIIT and CT groups, although less so in older CT.

(C) Mitochondrial respiration per mitochondrial protein content was lower at baseline in older people.

(D) Mitochondrial respiration per mitochondrial protein content did not change with training.

(E) The fractional synthesis rate of mitochondrial proteins (Mito FSR) was measured as the incorporation of $[^{13}C_6\ ring]$-phenylalanine into mitochondrial proteins isolated from serial muscle biopsies. Mito FSR was similar between age groups at baseline.

(F) Mito FSR increased with HIIT in both age groups and then with RT and CT in the older group. Changes during sedentary (SED) control period were analyzed separately and included in graphs for comparison. Data from baseline comparisons are displayed as mean ± SD with p values for unpaired t test. Changes with training are presented as least square adjusted mean with Tukey HSD 95% confidence intervals with the horizontal dotted line set

at zero (no change from baseline). Within a training group, a difference between young and old is displayed as exact p value. Statistical significance from baseline is indicated as *p 0.05; **p 0.01; ***p 0.001. Exact p values are reported in Table S8.
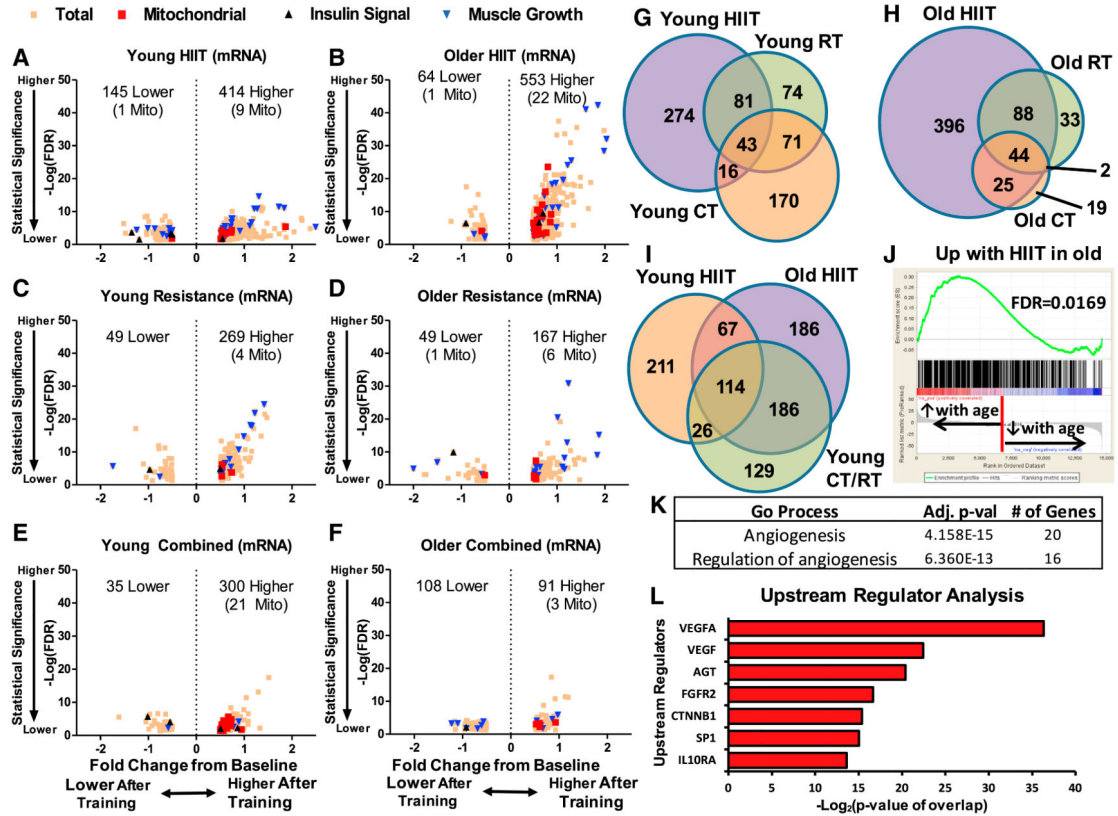
Figure 4. Muscle Gene Expression Changes with Exercise Training

(A–F) Genes that were differentially expressed following high-intensity interval training (HIIT) in the young (A) or older (B), resistance training in the young (C) or older (D), and combined training in the young (E) or older (F) using an adjusted p value of 0.05 and an absolute fold change of 0.5 were annotated according to their mitochondrial specificity (using MitoCarta) and molecular function (using KEGG). Mito stands for mitochondrial.

(G and H) Overlap of genes upregulated with different modes of exercise training in younger (G) and older (H) participants.

(I) Overlap of genes that were upregulated with HIIT in older adults and any type of exercise training in younger adults.

(J) Gene set enrichment analysis of baseline gene expression differences between young and old participants against genes that were upregulated with HIIT in older participants. Genes that increased expression with age were more likely to increase their expression with HIIT in older participants.

(K) A "universal exercise training response gene set" was derived by looking for genes that increased with exercise with an adjusted p value of 0.05 and an absolute fold change of 0.3 in all groups. Gene Ontology (GO) process annotations enriched for this universal exercise training response gene set was derived using MetaCore software configured with an adjusted p value threshold of 0.05.

(L) Ingenuity Pathway Analysis (QIAGEN) was used to detect up stream regulators of the "universal exercise training response gene set."
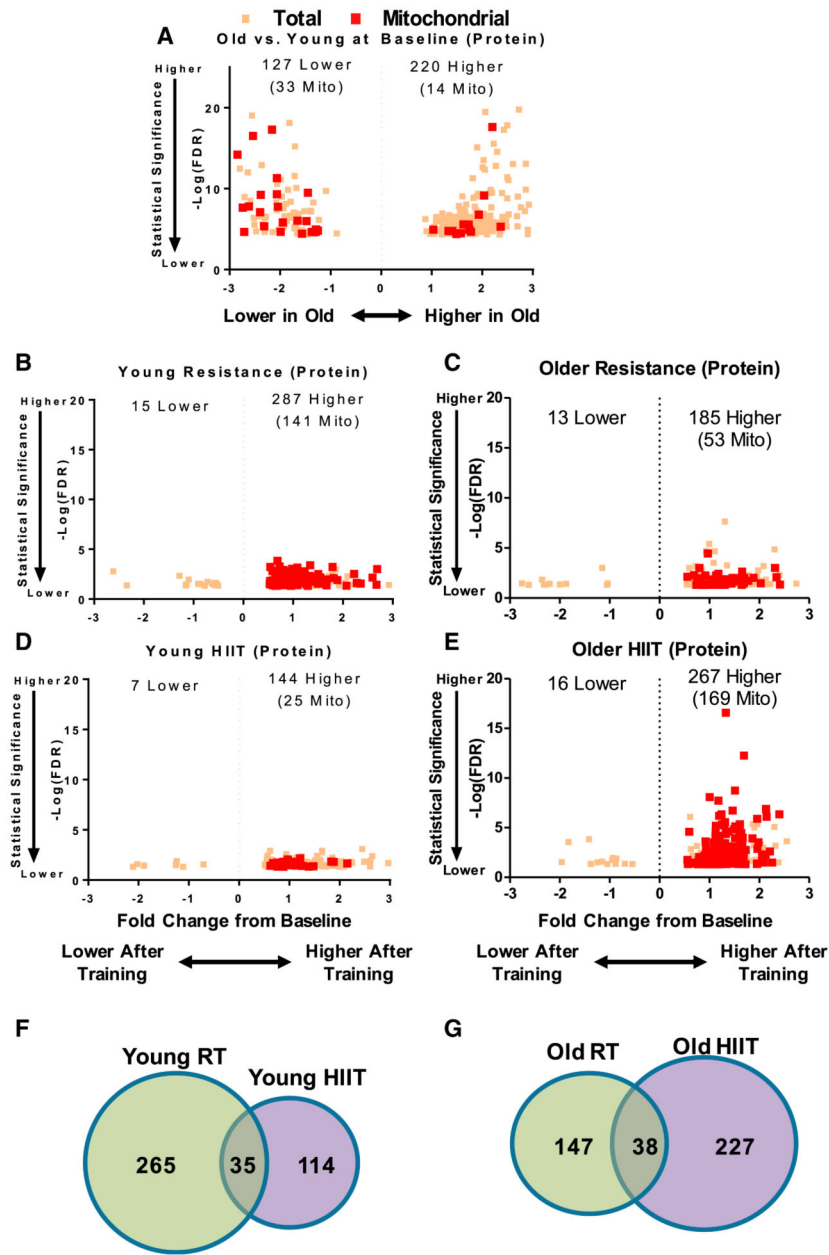
Figure 5. Muscle Protein Abundance with Age and Changes with Exercise Training
(A–E) Baseline differences in muscle protein abundance between young and older adults revealed decreased expression of 33 mitochondrial proteins (A). MaxQuant software configured to process label-free data was used to detect differentially expressed proteins with an adjusted p value of 0.05 and an absolute fold change of 0.5 following resistance training in the young (B) or older (C) or high-intensity interval training (HIIT) in the young (D) or older (E). MitoCarta database was used to highlight mitochondrial proteins. Fold change in skeletal muscle protein expression following 12 weeks of resistance (RT) or high-intensity aerobic interval training (HIIT) in young and older adults (B–E). Mitochondrial

protein abundance increased in both RT and HIIT modalities with pronounced gains following HIIT in older adults.

(F and G) Overlap of proteins that increased abundance with RT and HIIT in younger (F) and older (G) subjects, respectively.
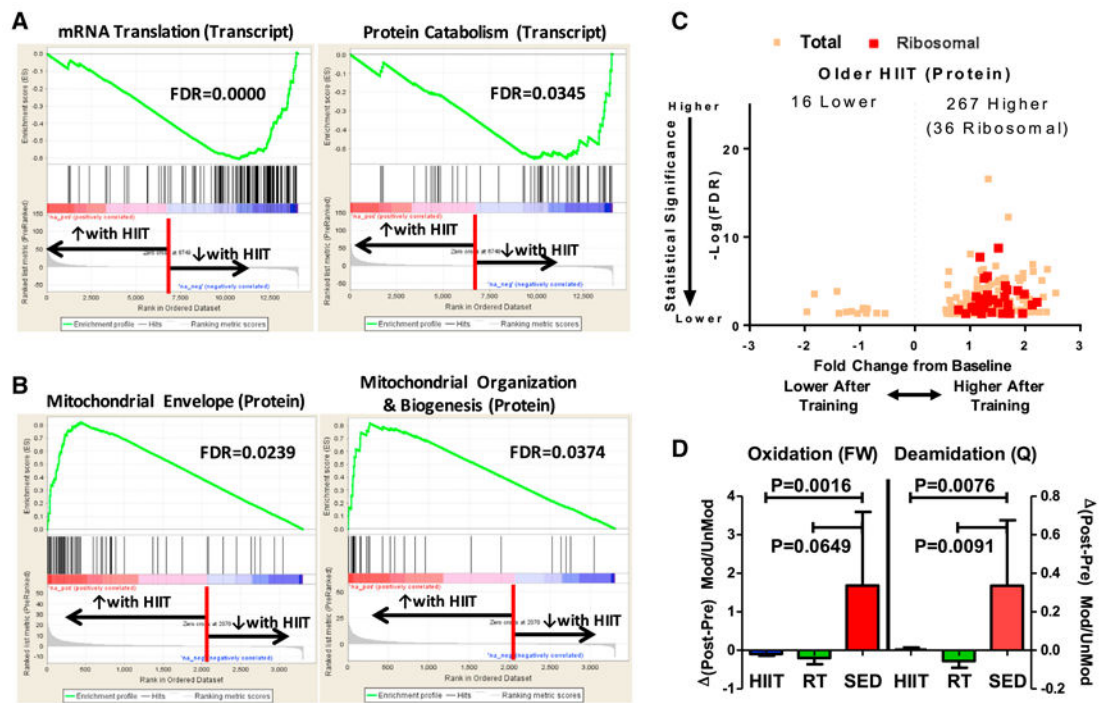
Figure 6. Downregulation of mRNA while Protein Abundance Increased for Mitochondrial and Ribosomal Protein with HIIT in Older Adults

(A) Gene set enrichment analysis of transcripts reveal downregulation ofmRNA for translational and protein catabolism pathways between pre-HIIT and post-HIIT in older cohort (adjusted p value < 0.05 and an absolute log2 fold change 0.5).

(B) Gene set enrichment analysis of proteins demonstrate an increased abundance of mitochondrial proteins between pre-HIIT and post-HIIT in older cohort (adjusted p value < 0.05 and an absolute log2 fold change 0.5). The software was configured to find enrichment from predefined gene sets in MSigDB.

(C) Ribosomal protein abundance increased with HIIT in older, supporting a role for greater translational capacity. MaxQuant software configured to process label-free data from older HIIT group was used to detect differentially expressed proteins with an adjusted p value of 0.05 andan absolute fold change of 0.5. Mitochondrial ribosomal proteins are highlighted in red and detailed in Table S7.

(D) Post-translational modification analysis of older subjects showed decreased oxidative damage to proteins after exercise training when compared to sedentary (SED) state. For this, MS1 intensities of peptides with phenylalanine oxidation, tryptophan oxidation, and glutamine deamidation were used to compute the ratio of modified (Mod) to unmodified (UnMod) quantities. Distribution of ratio of Mod/UnMod for all peptides was plotted and (two-sided) p values were computed using Mann-Whitney rank-sum test.

Table 1

Baseline Differences between Young and Older Participants

	Young			Older			ANOVA p value		
	HIIT	Resistance	Combined	HIIT	Resistance	Combined	Age	Group	Age × Group
N	14 (7 M/7 F)	11 (5 M/6 F)	9 (5 M/4 F)	9 (4 M/5 F)	9 (5 M/4 F)	8 (5 M/3 F)	N/A	N/A	N/A
Age	25.4 y (4.3 y)	23.7 y (3.5 y)	26.3 y (2.7 y)	70.7 y (4.6 y)	70.3 y (3.9 y)	68.6 y (3.4 y)	N/A	N/A	N/A
Height	174.5 cm (6.9 cm)	172.3 cm (14 cm)	173.4 cm (8.7 cm)	170.7 cm (10.7 cm)	168.9 cm (10.8 cm)	169.5 cm (9.7 cm)	0.1458	0.8745	0.9987
Weight	75 kg (9.7 kg)	73.8 kg (14.3 kg)	77.9 kg (17.1 kg)	80.3 kg (17 kg)	76.4 kg (13.7 kg)	77 kg (16 kg)	0.5534	0.8475	0.8041
BMI	24.6 kg/m^2 (2.3 kg/m^2)	24.7 kg/m^2 (2.7 kg/m^2)	25.6 kg/m^2 (3.3 kg/m^2)	27.3 kg/m^2 (3.3 kg/m^2)	26.6 kg/m^2 (2 kg/m^2)	26.6 kg/m^2 (3.8 kg/m^2)	0.0138	0.8377	0.6606
Body Fat	33.5% (7.1%)	28.3% (8.9%)	31.9% (4.7%)	36.2% (4.6%)	38.5% (4.5%)	37.9% (6.1%)	0.0002	0.5553	0.1088
Fasting Insulin	5.6 µIU/mL (2 µIU/mL)	5.7 µIU/mL (3.8 µIU/mL)	5.5 µIU/mL (2.6 µIU/mL)	5 µIU/mL (3.1 µIU/mL)	5.8 µIU/mL (2.1 µIU/mL)	4.3 µIU/mL (1.8 µIU/mL)	0.5698	0.8185	0.4807
Fasting Glucose	97 mg/dL (4 mg/dL)	96 mg/dL (6 mg/dL)	96 mg/dL (7 mg/dL)	104 mg/dL (11 mg/dL)	103 mg/dL (10 mg/dL)	105 mg/dL (8 mg/dL)	0.0003	0.6446	0.9092

Body composition was measured by dual energy X-ray absorptiometry. Data are mean with SD. BMI, body mass index; FFM, fat-free mass; HIIT, high-intensity interval training; N/A, not applicable.